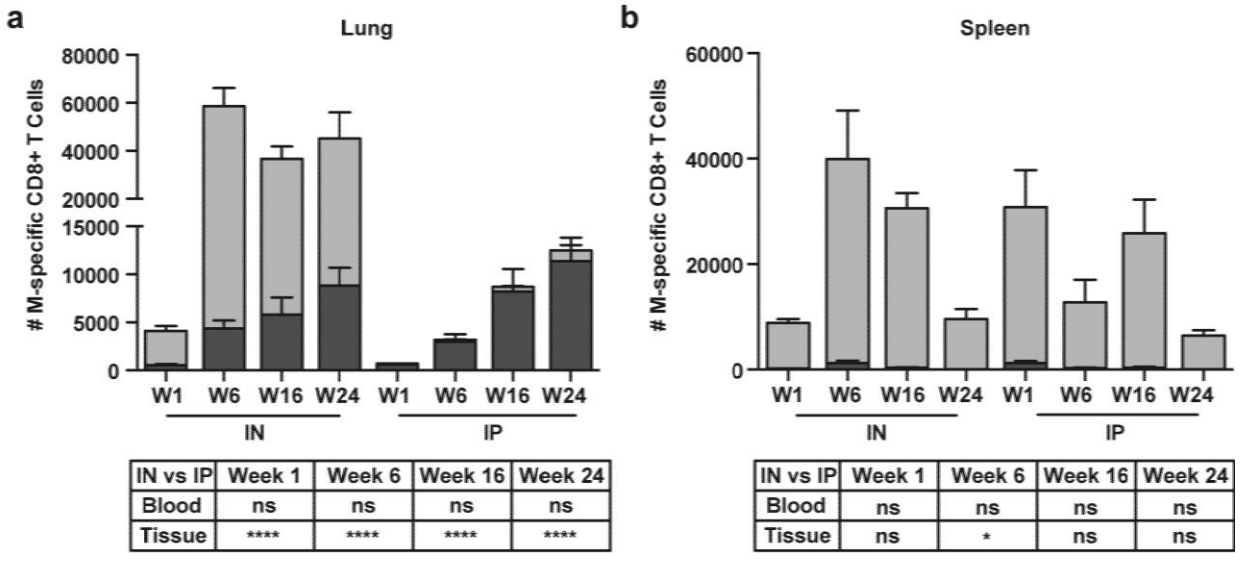
Supplementary Figure 1. IN vaccination generates more M-specific CD8+ T cells in the lung parenchyma than IP vaccination. At weeks 1 (W1), 6 (W6), 16 (W16), and 24 (W24) post-vaccination with MCMV-M via the IN or IP route, mice were injected IV with anti-CD45 antibody 5 minutes prior to sacrifice to identify cells in the blood (black) or tissue (grey). M-specific CD8+ T cells were identified by tetramer staining and flow cytometry. **(a**, **b)** Number of M-specific CD8+ T cells in the tissue and blood of the lungs (a) and spleen (b). Bars represent mean ± SEM with 5 mice per group. ****$p<0.0001$ and *$p<0.05$ by two-way ANOVA with Tukey's post-test for multiple comparisons. Data represent two independent experiments.

Supplementary Figure 2. IN vaccination with MCMV-M augments the M-specific CD8+ T cell response via the induction of effector and effector memory cells in the lung parenchyma and blood. The memory phenotype of M-specific CD8+ T cells harvested from the indicated sites was determined at week 16 and week 24 post-vaccination with MCMV-M. Mice were injected IV with anti-CD45 antibody 5 minutes prior to sacrifice to identify cells in the blood or tissue. Total number of central memory (CM), effector memory (EM), effector (E), and KLRG-1^+ effector (KLRG-1+) cells in the lung at week 16 and 24 post-vaccination. Bars represent mean ± SEM with 5 mice per group. **** $p \leq 0.0001$, *** $p < 0.001$, **$p < 0.01$, and *$p < 0.05$ by two-way ANOVA with Tukey's post-test for multiple comparisons. Data represent two independent experiments.

Supplementary Figure 3. IP administration of anti-CD8 antibody depletes CD8+ T cells from the tissue and blood of the lung, mediastinal lymph node, and spleen. At 16-weeks post-vaccination, mice vaccinated with MCMV-M by IN or IP route were treated with anti-CD8 antibody for 3 days prior to RSV challenge. On day 5 post-challenge, RSV viral lung titers were determined by plaque assay. On the day of RSV challenge, the number of CD4+, CD8+, and M-specific CD8+ T cells in the (a) lungs, (b) mediastinal lymph node and (d) spleen of mice treated with either anti-CD8 antibody or an isotype control for 3 days. (c) Number of CD8+ T cells and M-specific CD8+ T cells in the blood and tissue of the lungs identified by intravascular staining on day of RSV challenge. (e) FACS plots showing the percentage of CD4+ and CD8+ T cells gated on CD3+ cells (top panel) and the percentages of M-specific cells in the tissue and blood by intravascular staining gated on CD8+ T cells (bottom panel). Representative plots shown on day of RSV challenge, and on day 5 post-RSV challenge of mice treated with anti-CD8 antibody or an isotype control. Bars represent mean ± SEM with 5 mice per group for lung and spleen and 2 pooled samples of 2-3 mice for mediastinal lymph nodes. **** $p \leq 0.0001$ by two-way ANOVA with Tukey's post-test for multiple comparisons.

Supplementary Figure 4. FTY720 treatment reduces the number of CD62L+ CD8+ T cells in the blood of the lungs. 16-weeks after vaccination with MCMV-M by IN or IP route, mice were treated with FTY720. The total number of M-specific CD8+ T cells in the blood and tissue of the lung on the day of RSV challenge (a) and day 5 post-RSV challenge (e) identified by intravascular staining. On the day of RSV Challenge, the number of CD62L+ M-specific CD8+ T cells in the blood or tissue of (b) lungs, (c) mediastinal lymph node, or (d) spleen identified by intravascular staining. On day 5 post-RSV challenge, the number of CD62L+ M-specific CD8+ T cells in the blood or tissue of (f) lungs, (g) mediastinal lymph node, or (h) spleen identified by intravascular staining. Bars represent mean ± SEM with 5 mice per group for lung and spleen and 2 pooled samples of 2-3 mice for mediastinal lymph nodes. **** $p \leq 0.0001$, ** $p < 0.01$, $p < 0.05$ by two-way ANOVA with Tukey's post-test for multiple comparisons.

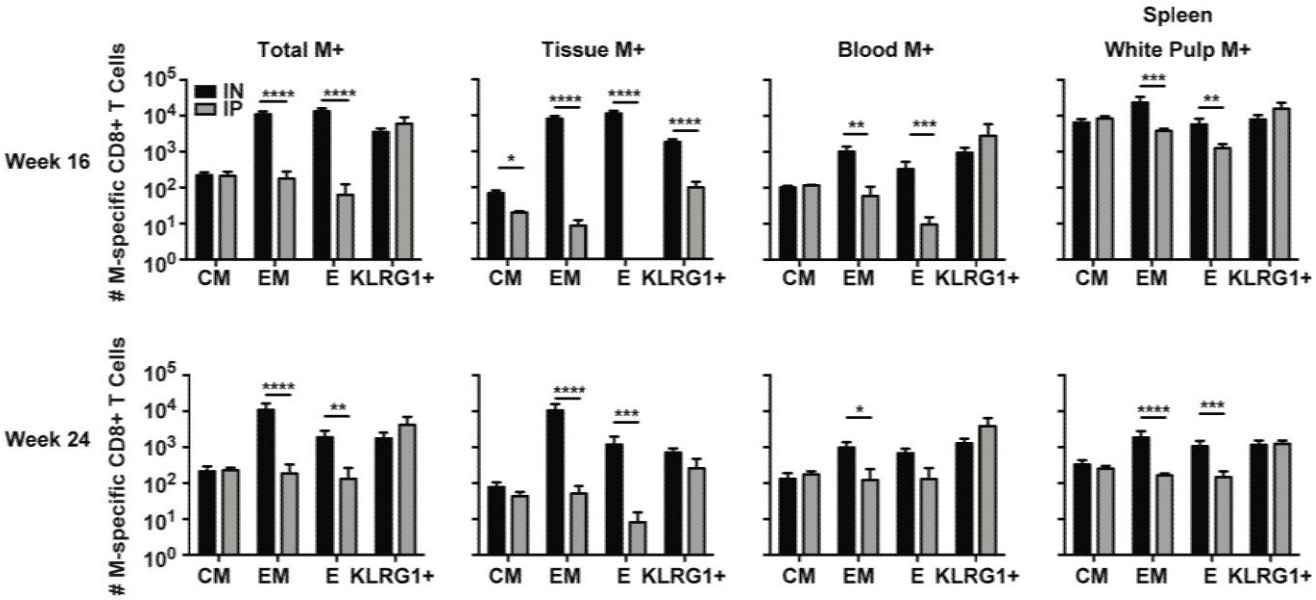
Supplementary Figure 1



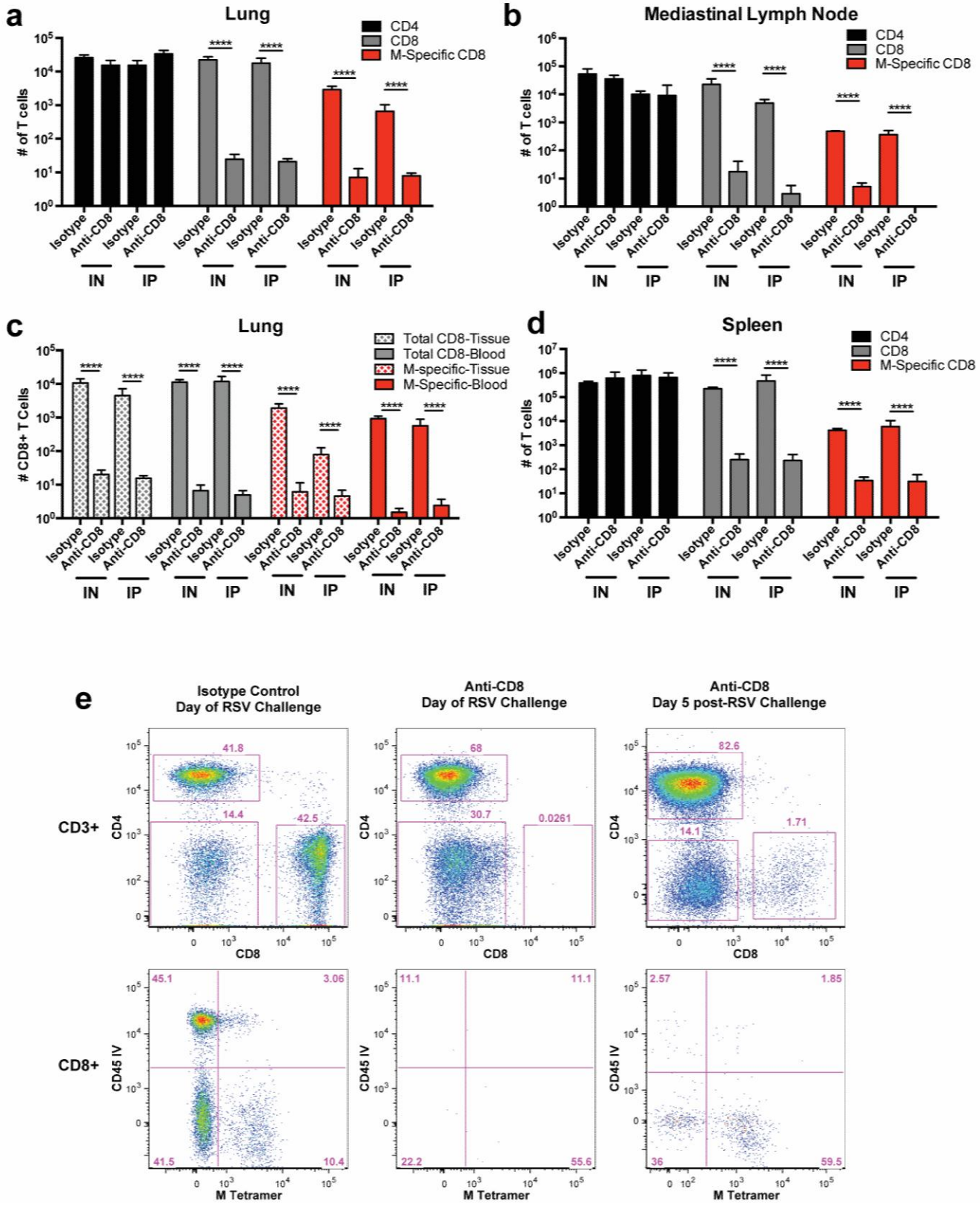
IN vs IP	Week 1	Week 6	Week 16	Week 24
Blood	ns	ns	ns	ns
Tissue	****	****	****	****

IN vs IP	Week 1	Week 6	Week 16	Week 24
Blood	ns	ns	ns	ns
Tissue	ns	*	ns	ns

Supplemental Figure 2



Supplementary Figure 3



Supplementary Figure 4